CLAIMS

- 1. A method of preparing an immunogenic membrane vesicle comprising isolating or purifying a membrane vesicle from a biological sample and contacting the membrane vesicle with a peptide or a lipid under conditions allowing the peptide or lipid to bind an antigen-presenting molecule at the surface of the membrane vesicle.
- 2. The method of claim 1, wherein the membrane vesicles are isolated from a biological sample comprising antigen-presenting cells.
- 3. The method of claim 2, wherein the membrane vesicles are isolated from a biological sample comprising human dendritic cells.
- 4. The method of claim 1, further comprising the step of subjecting the isolated or purified membrane vesicles to a selected acid medium prior to, during, or after contacting said vesicles with said peptide or lipid.
- 5. The method of claim 1, wherein the vesicles are subjected to density centrifugation or diafiltration to remove unbound peptide or lipid after the contacting step.
 - 6. The method of claim 1, wherein the peptide is a class-I restricted peptide.
 - 7. The method of claim 1, wherein the peptide is a class-II restricted peptide.
- 8. The method of claim 1, wherein the vesicles are contacted with a mixture of peptides.
- 9. The method of claim 8 wherein the vesicles are contacted with a peptide eluate of tumor cells.

- 10. The method of claim 1, wherein the antigen-presenting molecule is a CD1 molecule and the lipid is selected from a microbial lipid, a microbial glycolipid and a lipid or glycolipid tumor antigen.
- 11. The method of claim 1 wherein the immunogenic membrane vesicle comprises a complex of an exogenous class-I peptide bound to an HLA class I molecule, further comprising (i) subjecting an isolated or purified membrane vesicle to a selected acid medium, (ii) contacting the membrane vesicle with a class I-restricted peptide in the presence of beta2-microglobulin, under conditions allowing the peptide to complex with an HLA class I molecule at the surface of the membrane vesicle, and (iii) collecting the membrane vesicle.
- The method of claim 11, wherein step (i) comprises subjecting the membrane vesicles to a medium at a pH comprised between about 3 and about 5.5 for less than 15 minutes.
- 13. The method of claim 11, wherein step (ii) comprises contacting the membrane vesicle with 0.005 to 50 μ g/ml of class I-restricted peptide in the presence of beta2-microglobulin.
- 14. The method of claim 1 wherein the immunogenic membrane vesicle comprises a complex of an exogenous class-I peptide bound to an HLA class I molecule, further comprising (i) contacting the membrane vesicle with a class I-restricted peptide in the absence of beta2-microglobulin, (ii) subjecting the membrane vesicle to a selected acid medium under conditions allowing the peptide to exchange with any endogenous peptide for binding with an HLA class I molecule at the surface of the membrane vesicle, (iii) neutralizing the medium to stop the exchange and stabilize the complex formed in (ii) and, (iv) collecting the membrane vesicle.

- 15. The method of claim 14, wherein step (ii) comprises subjecting the membrane vesicle to a selected acid medium at a pH comprised between about 4 and about 5.5. for less than 2 hours.
- 16. The method of claim 14, wherein step (i) comprises contacting the membrane vesicle with 5 to 500 μg/ml of class I-restricted peptide in the absence of beta2-microglobulin.
- 17. The method of claim 11, wherein the peptide is selected from the group consisting of a tumor antigen, a viral antigen, a parasite antigen and a bacterial antigen.
- 18. The method of claim 14, wherein the peptide is selected from the group consisting of a tumor antigen, a viral antigen, a parasite antigen and a bacterial antigen.
 - 19. A method of preparing peptide-loaded membrane vesicles, comprising:
 - a) culturing of a population of antigen-presenting cells under conditions allowing the release of membrane vesicles by antigen-presenting cells,
 - b) purifying or enriching the membrane vesicles, and
 - c) contacting the membrane vesicles with a peptide under conditions allowing the peptide to bind an MHC molecule at the surface of the membrane vesicles to produce peptide-loaded membrane vesicles.
- 20. The method of claim 19 wherein the cultured population antigen-presenting cells are dendritic cells.
- 21. The method of claim 19, wherein the membrane vesicles are subjected to a selected acid medium.

- 27. The method of claim 24, wherein the peptide is a class-I restricted peptide.
- 28. The method of claim 24, wherein the antigen-presenting molecule is a CD1 molecule and the lipid is selected from the group consisting of a microbial lipid, a microbial glycolipid, and a lipid or glycolipid tumor antigen.
- 29. A pharmaceutical composition comprising an immunogenic membrane vesicle and a pharmaceutically acceptable diluent or carrier, wherein the immunogenic membrane vesicle is obtained by isolating a membrane vesicle from a biological sample containing antigenpresenting cells and loading said isolated membrane vesicle with an immunogenic peptide or lipid.
- 30. The pharmaceutical composition of claim 29, wherein the immunogenic membrane vesicle is obtained by isolating a membrane vesicle from a biological sample containing antigen-presenting cells, loading said isolated membrane vesicle with an immunogenic peptide or lipid and removing unbound immunogenic peptide or lipid by density centrifugation or diafiltration.
- 31. The pharmaceutical composition of claim 29, wherein the immunogenic membrane vesicle is obtained by isolating a membrane vesicle from a biological sample containing dendritic cells.
- 32. The pharmaceutical composition of claim 31, wherein the immunogenic peptide is a class-I restricted peptide.

20

5

- 22. A method of preparing peptide-loaded membrane vesicles, comprising:
 - a) obtaining a population of immature dendritic cells
 - b) culturing the population of immature dendritic cells under conditions allowing the release of membrane vesicles by immature dendritic cells,
 - c) purifying or enriching the membrane vesicles, and
 - d) contacting the membrane vesicles with a peptide under conditions allowing the peptide to bind an MHC molecule at the surface of said membrane vesicles to produce peptide-loaded membrane vesicles.
- 23. The method of claim 22, wherein the membrane vesicles are subjected to a selected acid medium.
- 24. A method of producing an immune response in a subject, the method comprising (i) obtaining a biological sample comprised of dendritic cells, (ii) isolating or purifying a membrane vesicle from said biological sample, (iii) contacting the membrane vesicle with a peptide or a lipid under conditions allowing the peptide or lipid to bind an MHC or CD1 molecule at the surface of said membrane vesicle, and (iv) administering the membrane vesicle to the subject to produce an immune response.
- 25. The method of claim 24, wherein, in step (i), the biological sample containing dendritic cells is obtained from the subject to be treated.
- 26. The method of claim 24, wherein the membrane vesicles are subjected to mild acid treatment.

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- 33. The pharmaceutical composition of claim 29, wherein at least 15% of HLA molecules at the surface of the vesicles are loaded with the peptide.
- 34. The pharmaceutical composition of claim 29, wherein at least 40% of HLA molecules at the surface of the vesicles are loaded with the peptide.
- 35. A method of preparing a pharmaceutical product comprising an immunogenic membrane vesicle and a pharmaceutically acceptable diluent or carrier comprising (i) isolating a membrane vesicle from a biological sample, (ii) loading the isolated membrane vesicle with an immunogenic peptide or lipid to produce an immunogenic membrane vesicle, and (iii) contacting the immunogenic membrane vesicle with a pharmaceutically acceptable diluent or carrier.
- 36. The method of claim 35 further comprising the step of removing unbound immunogenic peptide or lipid.